Pacific Science (1993), vol. 47, no. 1: 86–97

ABSTRACTS OF PAPERS

Seventeenth Annual Albert L. Tester Memorial Symposium,
15–16 April 1992[1]

The Albert L. Tester Memorial Symposium is held in honor of Professor Albert L. Tester, who, at the time of his death in 1974, was senior professor of zoology at the University of Hawaii. The faculty and students of the Department of Zoology proposed an annual symposium of student research papers as a means of honoring, in a continuing and active way, Dr. Tester's lively encouragement of student research in a broad range of fields in marine biology. Papers reporting original research on any aspect of biology are solicited from students at the university, and these papers are presented at the symposium, which takes place during the spring semester. Income from contributions to the Albert L. Tester Memorial Fund of the University of Hawaii Foundation is used to provide two prizes for the best papers by graduate students. Papers are judged on quality, originality, and importance of research reported, as well as the quality of the public presentation. Judges include several members of the faculty of the Department of Zoology as well as winners of the symposium from the preceding year, when possible. In addition, a distinguished scholar from another university is invited to participate in the symposium as a judge and to present the major symposium address. This year Robert Warner of the University of California, Santa Barbara, participated in the symposium.

Strategies of a Marine Parasite and Two of Its Hosts

G. S. Aeby[2]

Different species of parasites exhibit strategies that can range from maximizing to minimizing host exploitation, which in turn will select for different degrees of host defense. The strategies exhibited by a digenetic trematode, *Plagioporus* sp.; its coral intermediate host, *Porites* spp.; and a probable final host, a coral-feeding butterflyfish, were investigated to determine degree of host exploitation and host defense. Infected coral polyps appear as pink, swollen nodules on the colony and can no longer completely retract into their calices. Completion of the parasite's life cycle requires ingestion of infected polyps containing *Plagioporus* metacercariae by coral-feeding fishes. Degree of host exploitation was assessed by measuring the effect of parasitic infection on coral growth and the effect of altered polyp appearance on the level of predation on infected corals. Parasitic infection was costly to its coral host; infected corals had reduced growth when compared with uninfected corals, and corals exposed to fish predation had an increased loss of infected polyps suggesting increased predation. To determine whether altered appearance could be a host defense against the parasite, the effect of increased fish predation on growth of infected coral was assessed. Coral growth in predator exclusion

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cages did not differ from that of corals left exposed to predation, suggesting that altered polyp appearance could be a host strategy of parasitic defense. Degree of host defense within a final host was assessed by examining their prey choice. Laboratory studies showed that coral-feeding fishes preferentially fed on infected corals. Measurement of gut contents showed that fishes feeding on infected corals obtained more coral tissue per bite and thus had increased foraging efficiency. These results suggest that within the coral host, parasitism is costly and that corals could be responding by increasing risk of predation on infected polyps, but within the fish there was no evidence of host defense, suggesting that the cost of parasitism within this host may be minimal.

Activity Patterns and Home Range of the White Eel, *Conger cinereus*, and Two Proximate Cues Controlling Activity Initiation

REKA DOMOKOS[3]

In this study, I used underwater telemetry to determine the activity patterns and home range of the conger eel *Conger cinereus*. I also explored the possible role of three proximate cues in controlling the activity initiation of these eels: (1) satiation level of the eels, (2) environmental light intensity, and (3) time of day. Results of this study demonstrate that *C. cinereus* is a nocturnal species with an intermittent activity pattern alternating nights of activity with inactive nights. Each individual appears to have set patterns of activity that show a great deal of variation among eels but are relatively constant for each individual eel when active. Results of this study also indicate that this eel has a defined home range, that it may use more than one site for refuge, and that these sites may be shared with other animals in the eel's habitat. I found that two of the proximate cues explored in this study, satiation level of the eels and decrease in environmental light intensity, contribute to controlling the activity initiation of *C. cinereus*. Activity is initiated when the eels are motivated to forage by low satiation levels; however, the environmental light intensity must decrease below a specific level for the initiation of activity to occur.

Independent Effects of Ethanol, Acetaldehyde, and Acetate on Blood Pressure, Heart Rate, and Body Temperature after Alcohol Ingestion[4]

COLIN L. FAIRMAN[5]

Gas chromatography was used for simultaneous determinations of plasma ethanol, acetaldehyde, and acetate concentrations after the ingestion of 70 ml of 100-proof alcohol (0.477 g ETOH/kg weight) by six male volunteers, four of European and two of Asian ancestry. Volunteers arrived in the lab at 0900 hours and at 0930 hours were fitted with an indwelling catheter in the right subcubital vein. Alcohol was ingested at 1000 hours. Every 20 min for 6 hr blood samples (3 ml) were drawn and blood pressure, heart rate, and oral, axial, index finger, and ear temperatures were recorded. These results show rapid elevation of plasma ethanol levels and concomitant ap-

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[4] Supported in part by a grant from the University of Hawaii Project Development Funds.

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pearance and rise of acetaldehyde and acetate levels. Plasma ethanol levels peaked within 30 min and returned to zero by 6 hr, while acetaldehyde and acetate levels remained elevated for 6 hr. Each subject served as his own control. Changes in effects measured suggest a correlation with individual causal agents.

Ecological Genetics of Hawaiian Honeycreepers[6]

ROBERT A. FELDMAN[7]

We are investigating molecular genetic variability in common and rare species of the endemic Hawaiian honeycreepers (Fringillidae: Drepanidinae). The honeycreepers are one of the last spectacular examples of extreme adaptive radiation in an island avifauna and are highly variable in bill morphologies, plumage colorations, and behavioral and reproductive ecologies. Current lack of knowledge regarding their population dynamics limits conservation biologists hoping to ensure the long-term survival of this group. We are conducting mtDNA sequence studies on six species of honeycreepers, representing natural populations sampled nondestructively at Hakalau Forest NWR, Mauna Kea, on the island of Hawaii. We have also designed a PCR diagnostic test for *Plasmodium* that allows us to identify birds infected with malaria, quantify their levels of parasitemia, and investigate which introduced alien species may be serving as disease reservoirs. Our PCR-based detection system carries the advantage of strain identification, allowing new insights into the evolution of pathogenicity based on DNA sequence changes of *Plasmodium* rRNA genes. We hope that by linking our study of mtDNA genetic variability in the honeycreepers to the malaria diagnostic PCR tests for quantitating pathogen loads, we may provide essential information for zoos anticipating captive breeding programs and for naturalists planning mating experiments. Our goal is to produce an integrated strategy for the conservation of the Hawaiian honeycreepers.

Pair Bond Development in the Harlequin Shrimp, *Hymenocera picta*[8]

G. CURT FIEDLER[9]

Forty laboratory-reared *Hymenocera picta* juveniles, 2 weeks after the settlement stage, were randomly assigned to pairs. Individual pairs were placed in opaque containers with running seawater and aeration, and fed pieces of *Linckia* spp. ad libitum. Intra-pair distance (IPD) and development of external sex characteristics were recorded for 7 months. Average IPD was less for male/female pairs than for pairs of other sex combinations. For the 20 pairs, 10 differentiated into male/female pairs and 9 were male/male pairs. One female/female pair was observed. Using a 1 : 1 sex ratio based on field collections, a chi-square test for goodness of fit applied to the sex

[6] Funded by a MacArthur Foundation grant to L. Freed, R. Cann, and S. Conant.

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[8] Contributions to this work by S. Kraul of the Waikiki Aquarium and M. Lutnesky and J. Godwin of the Department of Zoology are appreciated.

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combination data indicated a significant deviation from expected counts. The data indicate that IPD may be used as a measure of pair bond formation. Further, a process such as environmental sex determination or protandry may be skewing the sex ratio observed in this experiment.

Intracellular Calcium as a Determinate of Outgrowth in Regenerating Crustacean Neurons in Defined Culture[10]

ROBERT A. GRAF[11]

Intracellular calcium levels $[Ca^{++}]_i$ have been proposed to influence the outgrowth of processes by neurons regenerating in culture. I examined identifiable cells from the crab (*Cardisoma carnifex*) eyestalk neurosecretory system (X-organ) cultured under defined conditions, for $[Ca^{++}]_i$ of neurons in relation to outgrowth. This project used videomicroscopy and computer-digitized ratioing of fluorescence intensity emission resulting from neurons that had taken up a Ca indicator, fura-2/AM, to quantitate $[Ca^{++}]_i$. I found that some of the neurons responded to stimulation by elevated $[K^+]_o$ (5X) with an immediate increase in the $[Ca^{++}]_i$ of the soma. Prestimulation levels ranged from 50 to 200 nM and stimulated levels rose at least 200 nM. Of neurons responding, those with extensive growth recovered toward prestimulation $[Ca^{++}]_i$ levels within 20 min, even in the continued presence of K, while $[Ca^{++}]_i$ remained high and recovered slowly and incompletely in those that exhibited little outgrowth. These results suggest that the capability for effective regulation of $[Ca^{++}]_i$ is important for outgrowth.

Interactive Effects of Temperature and Body Size on Fluorescent Age-pigment Variability in Fish[12]

KEVIN T. HILL[13]

Quantification of fluorescent age-pigment (FAP) metabolites has recently been proposed for estimating chronological age in aquatic organisms. The study reported here was conducted to determine whether temperature, ration, and body size can influence FAP content in postmitotic tissues and ultimately affect the ability to predict age. Cohorts of Rosy Barb (*Puntius conchonius*) juveniles reared at 19, 25, and 31°C, fed either ad libitum or restricted rations, had marked differences in soluble FAPs in brain and myocardial tissues after 6 months. Both whole-organ and weight-specific FAP content in brain and myocardium was inversely related to body size within and between treatments, presumably because of differences in mass-specific rates of oxygen consumption. Brain FAP was negatively correlated with temperature, independent of increased rates of oxygen

[10] Supported by NSF grant BNS-8910432, the Whitehall Foundation, and the University of Hawaii Foundation.

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[12] Supported in part by grants from the Sea Grant College Program, the Center on Aging, and a Biomedical Research Support Grant (to Christopher Womersley), and a Sigma Xi grant (to Kevin Hill).

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consumption at higher temperatures, suggesting involvement of other factors in their genesis. These factors may include temperature effects on the activity of antioxidant enzymes or the relative degree of unsaturation of cellular lipid components, leaving lipid-rich brain tissues more susceptible to peroxidative damage and FAP formation at lower temperatures. This raises questions as to the utilization of FAPs for predicting chronological age in heterogeneous populations subjected to wide variations in biotic and abiotic growth parameters.

Divergence in Foraging Behavior and Wing Design of the Hoary Bat[14]

DAVID S. JACOBS[15]

The insectivorous Hawaiian hoary bat, *Lasiurus cinereus semotus*, may have experienced divergence through character release after its isolation from the ancestral North American hoary bat, *L. c. cinereus*. *Lasiurus c. cinereus* coexists with a number of other insectivorous species of bats. This study investigated differences in foraging behavior and wing design. In contrast to the North American hoary bat, which utilizes only open habitats, the Hawaiian bat forages among vegetation as well as in open habitats. The Hawaiian bat is much smaller (56% of mass), so wing parameters were compared with allometric equations for bats in general. The Hawaiian bat has a larger wingspan, larger wing area, and lower wing loading than *L. c. cinereus*. This trend has been identified in other vertebrates as adaptations for slower, more maneuverable flight among vegetation. It has an aspect ratio (wingspan^2/wing area) comparable to that of *L. c. cinereus*. High aspect ratio has been shown to be correlated with fast, efficient flight in open areas. The Hawaiian bat has thus retained the ability for relatively rapid, efficient flight in open areas and has acquired greater maneuverability for foraging among vegetation.

Chromosome Number in Ten Species of the Coral Genus *Acropora*[16]

JEAN C. KENYON[17]

Chromosome number was determined for 10 species of the coral genus *Acropora* from Guam and Okinawa, using colchicine-treated cells of 8- to 11-hr-old embryos. Six species (*A. digitifera*, *A. monticulosa*, *A. surculosa*, *A. valida*, *A. nobilis*, and *A. squarrosa*) had 28 chromosomes. Chromosome numbers of 24, 30, 42, and 48, established for *A. irregularis*, *A. divaricata*, *A. azurea*, and *A. ocellata*, respectively, suggest a polyploid series with a basic number of $x = 6$. Polyploidy is an important mechanism of speciation in the plant kingdom, but is thought to be rare in animals. The ability to propagate clones through vegetative fragmentation plus opportunities for hybridization afforded by synchronized multispecies spawning may have contributed

[14] Supported by grants from the state Division of Forestry and Wildlife, Audubon Society, Hawaiian Telephone Company, and Alvin Y. Yoshinaga (P.I., S. Conant), and MacArthur Foundation (P.I., L. Freed; co-P.I.s, R. Cann, S. Conant).

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[16] Supported by the Charles H. and Margaret B. Edmondson Research Fund; the International Agreements Fund of the School of Hawaiian, Asian, and Pacific Studies; and a Research Assistantship from the Research Corporation of the University of Hawaii.

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to development of polyploidy and rapid speciation in this genus. Karyotypic data may aid in resolving some of the taxonomic confusion that surrounds this speciose genus.

Avian Community Changes after an Alien Plant Invasion[18]

MAILE S. KJARGAARD[19]

A dramatic proliferation of *Myrica faya* (Ait.) has occurred in Hawaii Volcanoes National Park during the last decade. To determine the effects of this invasion on the avian community, I repeated census and vegetation observations at 25 stations along a transect that had initially been sampled during the U.S. Fish and Wildlife Service Hawaiian Forest Bird Survey in 1979. Cover abundance and average size of *Myrica* have dramatically increased along the transect since the original observations. At that time, small *Myrica* were present at low densities at many of the stations, but this species was never a significant component of the canopy. Now, 10 of the stations have *Myrica* cover values at the canopy level of at least 25%. Census results show a marked reduction in the ratio of densities of native to alien bird species in the parts of the transect where *Myrica* cover has been allowed to increase. These changes are the result of both an increase in numbers of alien frugivores and greatly reduced densities of native species.

Enhanced Food Availability and the Achatinelline Tree Snail *Partulina redfieldii*[20]

SHARON R. KOBAYASHI[21]

The Hawaiian tree snails (subfamily Achatinellinae) are unique to the Hawaiian Islands and are highly endangered in the wild. Achatinellines are arboreal pulmonate gastropods characterized by slow growth and late age at first reproduction. The purpose of this research was to determine whether these unusual life-history characteristics are solely due to genetic factors or are at least partly environmentally determined. Juvenile *Partulina redfieldii* were kept in environmental chambers with conditions set to emulate those in native habitats. The snails were provided with fresh leaves and branches of *Metrosideros polymorpha*, a natural substratum for the snails, augmented by cultures of native fungi grown on potato dextrose agar. Comparison of snails in the laboratory with similar-sized snails in the field revealed significantly faster growth in the laboratory animals. Subsequently, laboratory comparisons of snails maintained with and without agar-grown fungi demonstrated that food availability is the major cause of limited growth rate in the field. However, even under optimal conditions, *P. redfieldii* exhibits a significantly lower growth rate than that observed for other arboreal snail groups, suggesting a genetic influence.

[18] Partial support provided by Hawaii Evolutionary Biology Program (HEBP), Ecology, Evolution, Conservation Biology (EECB) graduate program.

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[20] Supported by a grant to M. G. Hadfield from the Cooke Foundation.

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Effects of Suspension Feeding and Physical Disturbances on Oyster Reef Infauna

SCOTT THOMAS LARNED[22]

Consumption of settling larvae by dense populations of suspension-feeding invertebrates and habitat modification by physical disturbances are major determinants of ecological structure of some marine infaunal assemblages. The infauna of a naturally occurring oyster reef on the mudflats of Arcata Bay, California, is subject to effects of both processes: suspension feeding by dense populations of the native oyster *Ostrea lurida*, and excavation of pits by bat rays foraging for infauna. Two manipulative field experiments were conducted on the oyster reef to investigate the effects of these processes on infaunal structure and their relationships to the life histories of infaunal species. In the first experiment, patches of reef in which live oysters were replaced with equal volumes of dead oyster valves experienced heavy sediment deposition and had shallower oxidized layers, lower species richness, and lower infaunal densities than adjacent sites with live oysters. In the second experiment, simulated bat ray excavations were quickly filled by sediment transported in tidal currents and had shallow oxidized layers. The infauna of disturbed patches was strongly dominated by four species. Species composition in disturbed patches was more similar to that of the mudflats than to that of the undisturbed oyster reef. Common mudflat and disturbed reef patch species have planktonic development and dispersal, while common undisturbed reef species have direct development and juvenile and adult dispersal capabilities. Results of these experiments suggest that: (1) regulation of sediment flux and sediment oxygenation by oysters benefits infaunal organisms that avoid consumption during settlement; and (2) direct development and juvenile and adult dispersal contribute to the success of some oyster reef infauna, allowing such species to persist in a potentially hazardous suspension-feeding habitat.

Molecular Anthropology: Understanding Human Migration into the Pacific and Polynesia[23]

J. KOJI LUM[24]

Anatomically modern humans, *Homo sapiens sapiens*, colonized all continents by the late Pleistocene. Recent episodes of biological contact and interchange that occur when human populations expand into new regions provide information that is essential to reconstructing our species' more remote evolutionary past. Human expansion into Polynesia, over the last 5000 yr, gives researchers the opportunity to examine hypotheses of cultural and biological interchange that govern the way human societies interact. I report the discovery that Polynesians, considered a linguistically and culturally cohesive unit, contain evidence in their gene pool of two highly divergent maternal lineages, which probably last shared a common ancestor over 90,000 yr ago. The most likely explanation for this observation is that during the colonization of the Pacific, seafarers from Asia mixed with

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resident Melanesian peoples and carried a complex mixture of genotypes derived from these two distinct geographic sources throughout Polynesia.

A Nutritional Index for the Hawaiian Spiny Lobster, *Panulirus marginatus*[25]

THERESA L. MARTINELLI[26]

Crustaceans pose difficulties for gross assessment of nutritional state, in part because of their rigid exoskeleton and tendency to replace metabolized tissues with water. The Hawaiian spiny lobster, *Panulirus marginatus*, is a commercially important species, for which a reliable indicator of nutritional state may be valuable for assessing relationships between populations and habitats. Sensitivities of two known nutritional indices, gastric fluid protein content (GFPC) and muscle lipid content, were compared in a controlled experiment with Hawaiian spiny lobsters in several known nutritional states. Previous studies have shown GFPC to be an accurate index of nutritional state in a congener. Lobsters are known to maintain caudal lipid reserves that may correlate with period of starvation. One hundred male lobsters of similar size were collected and randomly assigned to experimental (starved) and control (fed ad libitum) groups. Throughout the simultaneous 25-day treatment period, lobsters were sacrificed from both groups at 5-day intervals. Although the GFPC showed significant differences between experimental and control groups and between initial and final levels within the experimental group, the variability was too high to allow accurate prediction of nutritional state. Pilot study data for muscle lipid content suggest a weak negative correlation with period of starvation.

Use of Nucleic Acid Ratios as an Index of Growth in the White Shrimp, *Penaeus vannamei*[27]

SHAUN M. MOSS[28]

RNA-DNA ratios have been used as indicators of somatic growth in a variety of organisms. This approach is based on the assumptions that RNA concentration varies directly with protein synthesis and DNA content per somatic cell is constant and thus reflects cell number. Rapidly growing organisms are expected to have higher RNA-DNA ratios than slow-growing organisms. The quantitative relationship between nucleic acids and growth in crustaceans is not well documented and may be complicated by physiological and behavioral changes associated with the molt cycle. Objectives of this study were to determine effects of molt stage on nucleic acid ratios in the white shrimp, *Penaeus vannamei*, and to compare nucleic acid ratios in fed versus starved shrimp to assess the usefulness of this ratio as an index of growth. Abdominal muscle tissue from shrimp in each of the five major molt stages (early postmolt, late postmolt, intermolt, early premolt, and late premolt) was analyzed for nucleic acids. Molt

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[27] Contributions to this work by R. Early are appreciated. This work was supported by an Edmondson Research Grant.

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stages were identified by assessing the degree of epidermal retraction from setal bases of the uropod and the development of new setae. RNA-DNA ratios ranged from 4.8 to 6.2 and were not significantly different among molt stages (ANOVA, $P < 0.05$). Shrimp fed ad libitum for 2 weeks had significantly greater RNA concentrations and RNA-DNA ratios than shrimp starved for 2 weeks, but starved shrimp had significantly greater DNA concentrations (t test, $P < 0.05$). These results indicate that nucleic acid ratios from abdominal muscle tissue in shrimp are unaffected by molt stage and may be useful indicators of growth in crustaceans.

A Nonmotile Transposon Mutant of *Listeria monocytogenes* with Pleiotropic Effects[29]

XUEMEI OU[30]

A nonmotile mutant of *Listeria monocytogenes* was generated by using the conjugative erythromycin resistance transposon Tn916ΔE. This mutant, which also had reduced hemolytic activity, possessed only one copy of the transposon. Revertants that recovered motility were identified on soft agar plates. One-third of the revertants were partial revertants that recovered motility but were still nonhemolytic. Moreover, complete revertants could be further divided into two classes: erythromycin-sensitive, in which no transposon could be detected, and erythromycin-resistant. However, southern blot results suggested that 80% of the partial revertants and those complete revertants that were resistant to erythromycin possessed more than one copy of the transposon. The positions of the transposon in these revertants were different from those in the mutant. The assumption based on these results was that the gene that was interrupted by Tn916ΔE is a regulatory gene. Currently, we are pursuing the cloning of the gene that has been targeted by the transposon insertion.

Arthropod Abundance and Community Structure Associated with *'Ōhi'a* (*Metrosideros polymorpha*) Canopy Foliage from Two Wet Forest Habitats Differing in Extent of Canopy Cover[31]

ROBERT W. PECK[32]

Hawaiian mesic and wet forests are dominated by relatively few canopy-forming tree species. *'Ōhi'a* (*Metrosideros polymorpha* Gaud.), the predominant tree, varies markedly in its growth form depending upon the extent of canopy cover. In closed-canopy areas, trees have a straight, polelike form, with foliage concentrated at the top, and form a nearly continuous forest canopy. In open-canopy areas, where *'ōhi'a* are widely spaced, trees become massive, with foliage extending fully from the top to halfway or more to the ground. These differences in forest structure, which may have arisen from events such as natural gap formation, logging, and cattle grazing, have led to a mosaic of forest types. This study investigated the community struc-

[29] Supported by the University of Hawaii; directed by Sophia Kathariou.

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[31] Supported by grants from Hawaii Audubon Society, Sigma Xi, and John D. and Catherine T. MacArthur Foundation (to L. Freed, R. Cann, and S. Conant).

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ture and relative abundance of arthropods associated with *'ōhi'a* canopy foliage from nearby areas that differ greatly in tree morphology and canopy cover in a montane forest on Mauna Kea, island of Hawaii. Over the course of 1 yr, foliage was sampled, and arthropods collected, via a pole-mounted branch clipper. In both forest types, spiders dominated the samples numerically, with psocopterans, homopterans, and hemipterans found in decreasing order. Greater spider biomass, leading to a greater overall biomass, was found in the open-canopy habitat. Because the study area represents primary habitat for canopy-foraging insectivorous birds, three of which are endangered, birds were censused contemporaneously with arthropod sampling. The two forest types are indistinguishable for these birds.

Growth and Development of Antarctic Larval Fish: Comparing Two Strategies[33]

JAMES J. RUZICKA[34]

The demersal fish of the Antarctic can be grouped into two larval development strategies based upon duration of the pelagic stage: short summer development and long multiseason development. Larval development was formerly believed to be short and limited to the productive summer season only (Marshall, 1953, Evolution 7:328–341). Kellermann, however, demonstrated that many larvae in fact have long pelagic stages and remain in the upper water column over winter (1986, Ber. Polarforsch. 31:149 pp.). The adaptive significance for each of these two strategies in terms of hatch period, growth, and development during the pelagic stage was examined. *Notothenia gibberifrons* is a species with single-season (summer) pelagic development. These larvae are hypothesized to grow and develop at a relatively rapid rate; they are able to achieve an ecologically viable settlement size and developmental stage by the end of the summer season. *Nototheniops larseni* is a species with multiseason pelagic development. Such a strategy is hypothesized to grant an extended period for growth and development that will allow larvae to settle at a more advanced stage and at a larger, more competitive size or compensate for an inability to grow and develop to a sufficient size and stage for settlement before the end of the summer. Larval fish collected from the Antarctic Peninsula and South Georgia were sized, their developmental state assessed, and otoliths removed for precisely aging fish and measuring growth rates. Techniques for examining the otolith microstructure were used to estimate hatch period, growth, and development during the pelagic stage. Both light microscopy and electron microscopy were used to control for artifacts of preparation and increment count estimation techniques. Light and electron microscopic methods of counting daily increments gave similar results, showing that light microscopy is sufficient for obtaining accurate counts without optical artifacts being a problem. Electron microscopy is, however, still necessary for revealing the initial increments deposited immediately outside the central core of the otolith that are otherwise not visible. Back calculation of hatch dates for *N. gibberifrons* ($n = 23$) from South Georgia showed that these fish hatch during a short period of about 26 days starting in mid- to late October. Back calculation of hatching period of *N. larseni* from South Georgia ($n = 10$) showed that these fish hatch over a period of at least 37 days, longer than that of *N. gibberifrons*. Examination of developmental state showed that the overwintering *N. larseni* develop slowly; more than 2 months after hatching, pectoral fin buds have not yet appeared and rays in other fins are still not fully developed. On the other hand, *N. gibberifrons*, which conclude their pelagic exis-

[33] Supported by NSF DPP-88-16521 (Richard Radtke).

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tence within a single season, are relatively well developed after 2 months in the plankton, having a complete set of fully developed fins. The hypotheses that *N. gibberifrons* is ecologically competent to settle at the end of the summer season and that *N. larseni* requires more time in the plankton are supported.

An Analog of Gonadotropin-releasing Hormone Stimulates Release of Prolactin and Inhibits Release of Growth Hormone from Pituitary Tissues of the Tilapia, *Oreochromis mossambicus*, in Vitro[35]

GREGORY M. WEBER, BUEL D. RODGERS, AND E. GORDON GRAU[36]

Prolactin (PRL) is an important reproductive hormone in most vertebrates. Nevertheless, the character of prolactin's involvement in teleost reproduction has received surprisingly little attention. Studies of PRL cell regulation associated with reproduction have also been limited. In mammals, exogenous gonadotropin release hormone (GnRH) treatment has been observed to raise or lower serum PRL levels, possibly dependent on basal PRL levels at time of treatment. Treatment of mammalian pituitaries with GnRH augments PRL release. This action is believed, however, to be induced through paracrine mediation by gonadotrophs. Studies of goldfish PRL cells failed to find GnRH receptors. We have examined the effects of GnRHa (des-Gly^{10}, [$_{D}$-Ala^{6}]-GnRH ethylamide) on PRL release from the rostral pars distalis (RPD) of the male tilapia, *Oreochromis mossambicus*, in vitro. Individual RPD, containing a nearly homogeneous population of PRL cells (95–99%), were incubated for 18–20 hr in media containing graded concentrations of GnRHa ranging from 0.01 to 1000 nM. During incubations in isosmotic (320 mOsmolal) and hyperosmotic (355 mOsmolal) media, GnRHa strongly stimulated the release of both forms of PRL, which differ by 11 amino acids. Release of PRL from cells, already highly stimulated by exposure to reduced osmotic pressure (300 mOsmolal), was not further stimulated by GnRHa. In parallel studies, the release of GH from the proximal pars distalis in vitro was inhibited by GnRHa at all osmotic pressures tested (300 mOsmolal, $P < 0.05$; 320 and 355 mOsmolal, $P = 0.01$). Overall, our findings suggest that GnRH may stimulate PRL secretion in the tilapia as it suppresses GH secretion. Our studies also suggest that the effects of GnRH on tilapia PRL cells are direct.

Environmental Salinity and Synthesis of the Two Prolactins of the Tilapia, *Oreochromis mossambicus*[37]

JOANNE S. M. YOSHIKAWA, RUSSELL J. BORSKI, AND E. GORDON GRAU[38]

Prolactin (PRL) is a freshwater (FW) osmoregulatory hormone in teleosts, including tilapia, *Oreochromis mossambicus*. Appropriately, PRL release is augmented within 10–20 min in vitro by physiological reductions in osmotic pressure (OP). The tilapia rostral pars distalis (RPD) synthesizes and releases two PRLs, encoded by separate genes and differing by 11 amino acids ($tPRL_{188}$ and $tPRL_{177}$). The growth effect of $tPRL_{188}$ on juvenile tila-

[35] Supported by NSF Grant DCB-9104494, NOAA Sea Grant No. NA89AA-D-SG063 R/AQ-37, and the Aquaculture Development Program (E. Gordon Grau).

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[37] Supported by NSF Grant DCB-9104494 and NOAA Sea Grant No. NA89AA-D-SB063 R/AQ-37 (E. Gordon Grau).

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pia is the only functional difference for these hormones yet described. Recently, we found that relative content of the two tPRLs in the RPD depends on salinity. The $tPRL_{188}$: $tPRL_{177}$ ratio is ~1.3 : 1 in FW and ~0.4 : 1 in seawater (SW). This suggests that the synthesis, release, and/or degradation of the two tPRLs may be separately regulated during shifts in salinity. Here, we have characterized the pattern of synthesis and release of the two tPRLs using RPD from fish acclimated to FW and SW. Male tilapia were held in FW or SW for 2 months. Individual RPD (95–99% PRL cells) were incubated with ^{3}H-leucine for 0, 40, 90, 150, 240, and 360 min. The tPRLs in media and tissues were separated by SDS gel electrophoresis. Incorporation of [^{3}H] into the tPRLs was used to assess synthesis and release. Synthesis of both [^{3}H]-tPRLs was greater and more rapid in RPD from FW tilapia than in RPD from SW fish. Reduced OP increased synthesis of the two tPRLs in RPD of both FW and SW tilapia. In FW, the increase in tPRL synthesis induced by reduced OP was not observed until 240 min. By 360 min, the ratio of [^{3}H]-$tPRL_{188}$: [^{3}H]-$tPRL_{177}$ was ~1.3 : 1 in FW tilapia RPD and ~0.5 : 1 in SW tilapia RPD. These findings, together with our previous work, suggest that the effect of OP on PRL release is at least partially independent of its action on PRL synthesis. Moreover, differential changes in the rates of synthesis of the two tPRLs may largely account for the reversal of the $tPRL_{188}$: $tPRL_{177}$ ratio in the tilapia RPD when fish are moved between FW and SW.